‘Self-fumigation’ of nests by an endangered avian host using insecticide-treated feathers increases reproductive success more than tenfold.

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Abstract

Parasites can cause great fitness cost to their hosts, however their impact on host populations is often unknown. In healthy populations, parasites are not expected to cause declines, but they can be devastating to small and/or declining populations. Nest ectoparasites can have detrimental impacts on the breeding output of their hosts and are emerging as a threat to several endangered bird species. Therefore, finding cost-effect ways to reduce the impact of parasites on endangered hosts is crucial. Although ‘close-order’ management techniques available to manage nest parasites are effective, they are often expensive and might not be suitable for species that are intolerant of intensive manipulation. We tested a low cost, ‘close-order’ management technique to control parasites and boost nest productivity in an endangered passerine. The endangered forty-spotted pardalote *Pardalotus quadragintus* is exploited by an ectoparasitic fly *Passeromyia longicornis*, an obligate subcutaneous parasite of nestling birds. We offered adult pardalotes the opportunity to ‘self-fumigate’ their nests by supplying feathers treated with insecticide with which to line their nests and tested whether this boosted nest productivity. Pardalotes readily incorporated the experimental feathers in nest building, and survival of hatchlings was significantly higher in nests lined with treated feathers (95%) compared to nests lined with control feathers (8%). This represents a substantially greater improvement in reproductive success than in previous experimental studies, offering the strongest evidence yet that self-fumigation is a highly effective, simple and low cost ‘close-order’ management technique for defending endangered birds against ectoparasites.

Keywords: forty-spotted pardalote, ‘close-order’ management, “self-fumigation”, parasitic fly, *Passeromyia longicornis*, endangered hosts, intensive management of endangered species, ectoparasites.

1. Introduction

Although parasites can cause great fitness cost to their hosts (e.g. reduced growth, survival and/or breeding success (Loye & Carroll, 1995; Tripet & Richner, 1997), host-parasite interactions are usually understudied when compared to other ecological interactions (e.g. predation and competition; Loye & Carroll, 1995), and their impact on host populations often remains unclear (Loye & Carroll, 1995; Combes, 2001). From an evolutionary perspective, parasites are not predicted to cause host population decline (Anderson & May, 1982). However, in declining and/or small populations parasites can become a threat (Heard et al., 2013). For example, in birds’ nests, ectoparasites can reduce reproductive success either by prolonging the nestling period, thereby decreasing the time available for re-nesting (as a consequence of reduced body condition in nestlings) or by increased probability of nest failure (Møller, 1993; Fitze, Tschirren, & Richner, 2004). In terms of lifetime reproductive success, parasitism can impact local recruitment because of the reduced number of fledglings per breeding attempt (Fitze, Tschirren, & Richner, 2004). These would not greatly affect healthy populations, but small populations are particularly vulnerable either because of their natural rarity (e.g. endemic populations restricted to islands), or their small size due to other driving factors (e.g. habitat loss, predation, competitors; Caughley, 1994; Heard et al., 2013; Cable et al., 2017).

In the Galápagos Islands, the introduced fly *Philornis downsi* has emerged as a threat to Darwin's finches. The larvae of *Philornis downsi* are nest ectoparasites that causes severe brood reduction and they have become the main cause of nestling mortality impacting recruitment in a species already in decline by other threatening processes (O'Connor et al., 2010; Cimadom et al., 2014)*.* Native *Philornis spp.* are also reported to have a major impact on the breeding success of several Neotropical bird species with small populations (Bulgarella, Quiroga, & Heimpel, 2019 Hayes et al., 2019). Given the impact parasites can have on small populations, there is a growing interest in the role of parasites and pathogens in conservation (Loye & Carroll, 1995; Smith, Acevedo-Whitehouse, & Pedersen, 2009). Moreover, finding management solutions to reduce the impact of parasites on endangered hosts becomes crucial particularly during such a vulnerable life stage (i.e. the nestling period), which can greatly impact small populations due to low recruitment.

In threatened species management, parasite control is undertaken using 'close-order' management techniques which focus on maximising fitness, i.e. survival and productivity at the individual level by targeting the factors that threaten them, and providing targeted management solutions in the wild (e.g. cross-fostering of birds, supplementary feeding; Bell & Merton, 2002; Jones & Merton, 2011). In 'close-order' management, control of ectoparasites is often achieved by manually adding insecticide to the nest substrate (e.g. black robin *Petroica traversi*, echo parakeet *Psittacula eques*, Jones, 2004). Adding beneficial nesting material (i.e. greenery with volatile chemicals) to nests might also be an option (Shutler & Campbell, 2007), and in species with extremely low numbers, intensive monitoring can be used to 'guard nests' and remove parasites (Bell & Merton, 2002). Although these techniques are effective, they are quite expensive and might not be feasible for some species (e.g. species that nest in small cavities or in inaccessible locations).

Moreover, funding for conservation is limited, so finding cost-effective ways to manage the impact of parasites may be crucial.

Taking advantage of species' behaviours might help us find creative ways to mitigate the impact of certain threats. For example, Knutie et al. (2014) provided cotton treated with insecticide to Darwin's finches during the nest building stage to be used as nesting material. This 'self-fumigation' experiment proved highly effective at reducing parasite load on nestlings. Here we investigate whether a similar approach could help improve the breeding success of another endangered host. We test the effectiveness of 'self-fumigation' as a low-cost, simple 'close-order' management technique that does not require substantial investment of time, money or personnel to reduce parasitism of the endangered forty-spotted pardalote *Pardalotus quadragintus* by an ectoparasitic fly, *Passeromyia longicornis*. Manual addition of insecticide to nests has been tested for forty-spotted pardadotes and proved to substantially improve breeding success (Edworthy *et al.* 2018). In this experiment, nests were sprayed a week prior hatching, and re-sprayed if parasites appeared on the nestlings. The results showed a substantial reduction of parasite intensity and higher fledging success in treated nests (Edworthy *et al.* 2018). However, this is not feasible as a long-term management practice, owing to the difficulty and expense of accessing the small cavities in high trees where forty-spotted pardalotes nest. Therefore, we tested whether pardalotes can be encouraged to "self-fumigate" their nests by carrying treated feathers up to their nest hollows as part of their natural nest building behaviour.

Forty-spotted pardalotes build fully domed nests inside tree cavities using tree bark, grass and soft material (usually feathers), which they use to line their nests (Wall, 1966; F. Alves *pers. obs*). We tested experimentally whether forty-spotted pardalotes could be encouraged to take insecticide-treated feathers to their nests, and whether this would reduce parasite load and boost reproductive success. Breeding pairs were provided with either control or treated feathers in dispensers near their nesting sites. We predicted that nests placed near treated feathers would have higher breeding output than nests near control feathers. We expected that with this technique we would boost productivity in pardalotes without the high resources and management manipulation often needed in intensive management strategies.

2. Material and methods

(a) Study species and site

Forty-spotted pardalotes are a small passerine endemic to Tasmanian forests where their preferred food tree, white gum (*Eucalyptus viminalis*) occurs. They are threatened by habitat loss and degradation, competitors, introduced species (Threatened Species Section 2006), and a recently discovered parasitic fly (Edworthy, 2016, Video S1). Forty-spotted pardalotes have become extinct across most of their former Tasmanian range (Brown 1986; Threatened Species Section 2006), and are now largely confined to two offshore islands (Bruny and Maria Islands). Forty-spotted pardalotes nest in tree hollows between August and January (Woinarski & Bulman, 1985). In 2012, larvae of the ectoparasitic fly *Passeromyia longicornis* (Diptera: Muscidae; Pont, 1974) were discovered in the nests of forty-spotted pardalotes (Edworthy *et al.* 2018). *P. longicornis* adults are free-living flies, and larvae are subcutaneous parasites of both native and introduced nestling birds (Pont 1974; Green and Munday 1971; Green 1988; Edworthy 2016). The larvae (Video S2) exploit 87% of forty-spotted pardalote nests in areas of high prevalence, and nestling forty-spotted pardalotes suffer 81% mortality in parasitised nests (Edworthy *et al.* 2018). The fly is endemic to Tasmania and so far has also been recorded parasitising four other host species (striated pardalote *P. striatus*, house sparrow *Passer domesticus*, New-Holland honeyeater *Phylidonyris novaehollandiae*, Edworthy 2016; Green and Munday 1971; Green 1988, and common starling *Sturnus vulgaris*; F Alves *pers. obs*). We conducted fieldwork on North Bruny Island (Latitude: -43° 09' 73.60" S, Longitude: 147° 35' 92.73" E) in a study site where prevalence of *P. longicornis* is known to be high (87% of nests, the same site where the previous elimination experiment was conducted; see Edworthy 2016 and Edworthy *et al.*

2018). The vegetation is dry forest dominated by *E. viminalis*, *E. globulus* and *E. pulchella* with a grassy understorey.

(b) Experimental set-up and nest monitoring

For two breeding seasons (August to January 2017 and 2018) we conducted a field experiment around a network of nest boxes known to be used by forty-spotted pardalotes (fig. S1). We followed the general protocol of a similar experiment conducted on Darwin's finches (*Geospiza, Camarhynchus* and *Platyspiza spp.*) on the Galápagos Islands (Knutie et al., 2014), but instead of using treated cotton we used treated feathers, as forty-spotted pardalotes line their nests with feathers. We placed sterilized store-bought chicken feathers in "feather dispensers" made of double hardware mesh to hold feathers in place, and attached a cover to the top of the dispensers to slow insecticide degradation from exposure to sunlight and rain (fig. 1). Control feather dispensers contained only untreated feathers, whereas treatment dispensers contained feathers sprayed with a commercial insecticide (safe to be used for birds; Avian Insect Liquidator: 1.25g/L Permethrin, 6.25g/L Piperonyl Butoxide, 20mg/L Methoprene). We re-applied insecticide to the treatment group every six weeks because after this period it starts to degrade. Dispensers were hung in trees at a mean distance of 4 m (SD = 1 m) from nest boxes in both control and treatment groups.

To control for an effect of site and/or season, we divided the study area in two halves; the half that received control "feather dispensers" in the first breeding season (2017) for the first clutch, received treatment for the first clutch in the second one (2018). Our experimental design also involved switching nests between treatment groups between each successive nesting attempt (pardalotes are multi-brooded) within a season. After a nest succeeded or failed we cleaned the nest boxes to encourage birds to rebuild and swapped their feather dispenser from control to treatment or vice versa. Our sites were laid out so that boxes were close to one another (~ 20 m between boxes), and all nests in an aggregation were switched between treatment groups simultaneously.

Nest initiation and success/failure dates were all highly synchronous, so it was possible to switch groups of nests between experimental treatments with no risk of overlap.

We located nests from the ground by observing nesting behaviour (i.e. territorial calls and birds carrying nesting material) and then we used single rope climbing techniques to monitor breeding success. We checked nests every four days at the beginning of a nesting attempt to determine clutch size and to estimate hatching date. Near the expected hatch-date we checked nests every three days to record more precise hatching dates and detect parasitism (nestlings usually die within five days after hatching if parasitised). We monitored 44 nests in 25 nest boxes over two breeding seasons (i.e. 2017 n= 11 control, 11 treatment; 2018 n=14 control, 8 treatment). We collected information on clutch size, brood size, presence/absence of experimental feathers in nests and presence/absence of *P. longicornis*.

Figure 1. “Feather dispenser” set up in the field (left). Feathers were glued to the base of the dispenser to increase the visual stimulus and encourage utilisation of feather dispensers. A small perch was provided on the dispenser to attract pardalotes. Nest (right) where pardalotes used the feathers.

(c) Data analyses

We recorded the average number of fledglings per nest (mean, SD) for each nest type (treatment or control). We used package “dabestR” (Ho et al., 2019) and built an estimation plot to visualise the effect size. We then used package “lme4” (Bates *et al*. 2015) and fitted a generalised linear mixed effect model to compare fledging success rates between control and treated nests. We used the number of nestlings that fledged vs. died as the response variable using a binomial distribution with nest type (treatment or control) and year (2017 or 2018) as a fixed effects. We also included nest box id as a random effect to account for the study design and multiple nesting attempts in a box. The analysis was conducted in R (R Core Team 2019).

3. Results

Four pairs attempted a second clutch in 2017 and six pairs in 2018. The breeding birds accessed the feather dispensers and used chicken feathers in nest building (Video S3) at 38 nests, whereas at six nests the pairs did not use the feathers. Nests without feathers were kept in the control group. Parasitic larvae were recorded in all control nests (mean ± SD larvae in each nest = 32.2 ± 9.7) and in three treated nests (mean ± SD larvae in each nest = 0.31 ± 0.8). Nests lined with treated feathers had substantially higher fledgling success (mean ± SD = 3.8 ± 0.9, Video S4) than control nests (mean ± SD = 0.3 ± 0.7; fig. 3). The survival rate (i.e. probability that a hatchling survived to fledge) was 8% (±SE = 3%) in control nests, compared with 95% (±SE = 3%) in treated nests. There was no effect of year in the model (table 1).

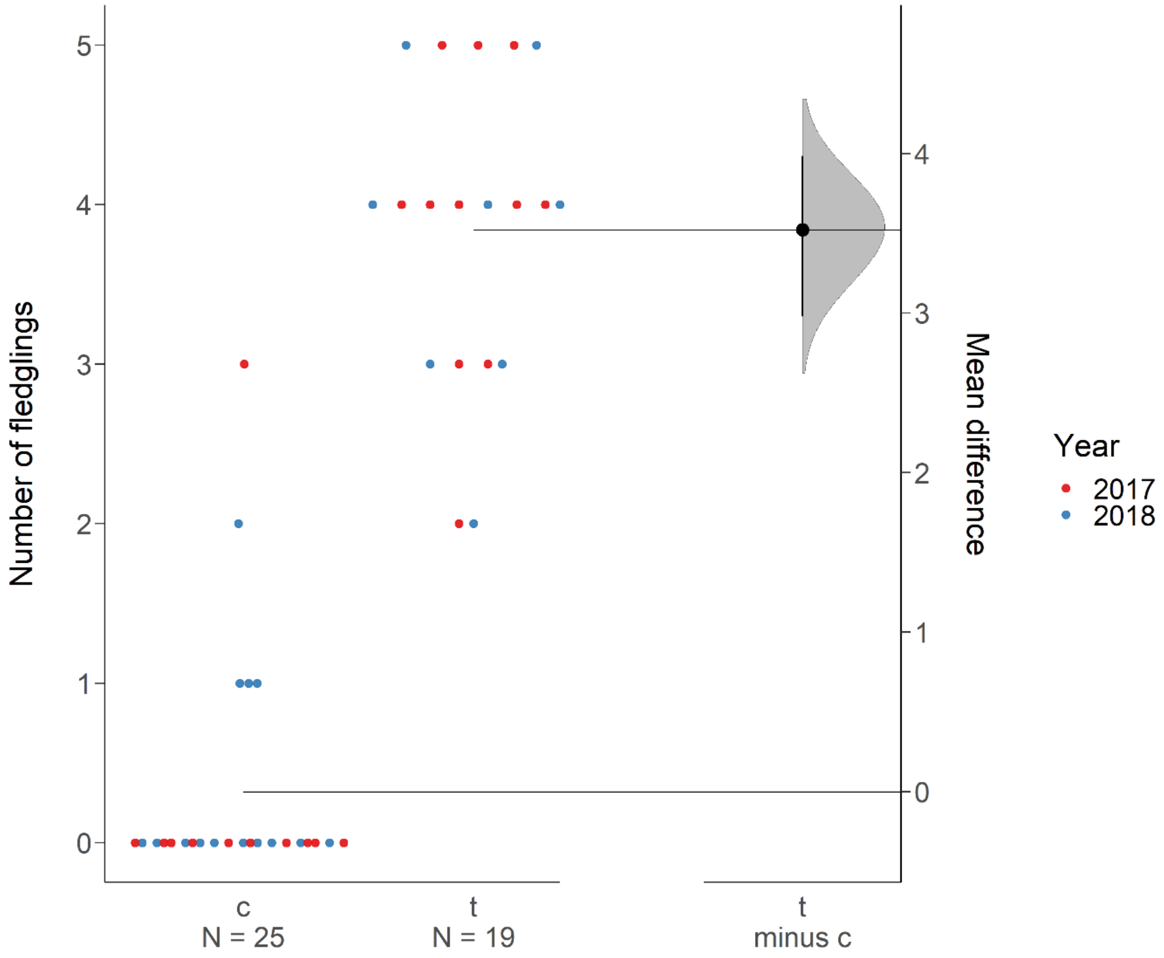

Figure. 3. Estimation plot showing the number of chicks that fledged (left y axis) in control versus treated nests. Right y-axis displays the mean difference and the bootstrapped-resampled distribution of the mean difference. The black vertical line shows 95% confidence interval of the mean difference (solid black circle), which is horizontally aligned with the mean of the treatment group.

Table 1. Estimated regression parameters, confidence intervals (CI), degrees of freedom (df) and P-values (p) for the Binomial GLMM fitted to the data.

	Proportion of nestlings that survived to fledge			
Predictors	*Odds Ratios*	*CI*	*df*	*p*
Intercept	0.09 ***	0.03 – 0.24	40.00	**<0.001**
Nest type (treatment)	208.30 ***	59.36 – 730.94	40.00	**<0.001**
Year (2018 vs 2017)	1.02	0.31 – 3.36	40.00	0.973

Random Effects	
$\tau_{00\ box\_id}$	0.00
N_{box_id}	25
Observations	44
Marginal R^2 / Conditional R^2	0.685 / NA
Deviance	59.931

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

The survival rate (i.e. probability that a hatchling survived to fledge) was 8% (±SE = 3%) in control nests, compared with 95% (±SE = 3%) in treated nests.

4. Discussion

Our study demonstrates that ‘self-fumigation’ may be used as a simple, cost-effective, ‘close-order’ management technique, resulting in immense improvements to reproductive success in endangered birds, whilst circumventing many of the disadvantages of more intrusive techniques. Our results might have implications for many bird species since parasitic flies are widespread and exploit a large range of hosts (Møller et al., 2009). “Self-fumigation” by forty-spotted pardalote proved to be a highly effective, simple and low cost means of substantially boosting fledging success; 95% of hatchlings survived to fledging in self-fumigated nests, compared to only 8% of hatchlings in untreated nests. This represents a greater increase in reproductive success than the previous experiment using self-fumigation (Knutie et al. 2014) or an experiment using manual spraying of nests (Edworthy *et.al.*, 2018). These results show that self-fumigation is a very promising conservation tool for species whose natural behaviours may be exploited for conservation management to increase productivity.

A previous experiment involving manual spraying of forty-spotted pardalote nests with insecticide resulted in 89% survival of hatchlings to fledging (Edworthy *et.al.*, 2018). Our self-fumigation experiment utilised a simpler methodology and yielded a 6% improvement in nestling survival compared to manually spraying nests. The survival rate of control nests in that experiment was the same as that found in our study (8%; Edworth*y et al.* 2018). Forty-spotted pardalotes that exploited feather dispensers for nesting material utilised chicken feathers heavily in nest construction (including both lining the nest cup itself but also as a more general construction material, Fig. 1). The higher survival rate found in our study might reflect the use of treated feathers as a nesting material within the nest interior, which is likely to be a more effective defence against parasitism than spraying outside the dome.

An experiment using manual spraying of the nests of Darwin’s finches increased fledging success from 54% to 83% of nestlings (Knutie et al. 2014). Knutie *et al.* (2014) also tested “self-fumigation” in Darwin’s finches by providing cotton treated with a 1% permethrin solution. While no information on fledging success was available for these nests, those containing treated cotton had a mean parasite load of 14.69 parasite larvae compared to 29.89 in control nests. In our experiment, only three treated nests contained parasites with a mean parasite load of 0.31, while all control nests contained parasites, with a mean parasite load of 32.2. Although the major differences between our study system and that of Knutie *et al.* (2014) precludes direct comparison, the lower parasite load we found in treated nests is likely to reflect a combination of insecticide concentration and nesting behaviour of pardalotes. Knutie et al. (2014) experiment used permethrin-treated cotton and we used a broad-spectrum parasite control spray for ornamental birds that contains Permethrin, Piperonyl Butoxide and Methoprene (Avian Insect Liquidator; Vetapharm, Wagga Wagga, New South Wales, Australia). Moreover, unlike Darwin’s finches, pardalotes nest in cavities where the treated nesting material is protected from degradation by sunlight and rain.

Not only does self-fumigation provide a more effective defence against parasites than manual spraying, it is also substantially more cost effective. Manual spraying requires significant time investment, in terms of both locating and then accessing nests in tree cavities, which often requires challenging tree climbing. By contrast, self-fumigation is simpler because feathers are deployed just above ground level, making tree climbing unnecessary. Construction of feather dispensers costs ~ $8 AUD/unit plus 1 h of assembly and deployment time. This low cost approach may feasibly be deployed at large enough scales to be suitable as a species conservation tool.

Furthermore, our dispensers and nesting material could be tailored to suit the ecology of many other bird species and nest-building mammals (e.g. deployment on the ground, use of different nesting material) which can also be afflicted by parasites (Hart & Hart, 2018). Self-fumigation could be easily adapted for any bird species that line their nests with soft material and even for species that do not use soft material, structural material (i.e. tree bark and grass) could be trialled. The only limitation we predict is that for species that nest in open cup nests insecticide degradation might happen faster, but even for those species minor reductions in parasite load might boost offspring survival.

Conservation actions are often criticised for not considering the importance of parasites to healthy ecosystems (Stringer & Linklater, 2014). However, in small and isolated populations, cumulative threatening processes (e.g. habitat loss, low availability of nesting sites) can exacerbate the impact of parasites, making parasite control justifiable (Stringer & Linklater, 2014). Nonetheless, parasites themselves are worthy of consideration from a conservation perspective (Gómez & Nichols, 2013). Observations of *P. longicornis* larvae exploiting the nestlings of four other bird species in Tasmania (see above) suggest that this parasite is a generalist. Thus in our case, control of parasitism in forty-spotted pardalote is unlikely to have a negative conservation impact on *P. longicornis* populations, because other, more numerous hosts remain available. There are currently no alternative methods available to control the impact of *P. longicornis* on forty-spotted pardalotes and this simple management intervention could be an effective way to mitigate this threat in the short term. Nest boxes have proven to be an important management tool for pardalotes given that most remaining populations are in second-growth forest where hollow availability is low, however providing nest boxes for pardalotes is not enough if nestlings have low chances of survival due to parasitism. Our experiment aimed to boost productivity at the individual level, but it is simple enough to be applied at larger scale and have an impact at the population level as well.

Identifying, testing and then rolling out new approaches to address the global extinction crisis and the diverse threats that are driving it are the central focus of conservation science. Our study shows that by exploiting the natural behaviours of a threatened species, innovative solutions may be created to overcome seemingly insurmountable conservation challenges. Approaches like the one we describe in this study are particularly important because they address the intensive, individual-level fitness limiting factors that can hinder population growth, but are also cheap and effective enough to be deployed at population and landscape scales with relatively low maintenance costs compared to other manual approaches to improving individual reproductive success.

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